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Impatiens walleriana, a new natural host of Amaranthus leaf mottle virus

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Abstract
Impatiens walleriana was introduced into Brazil and is now widely cultivated as an ornamental plant. Impatiens plants from a public garden showing leaf mosaic and colour break symptoms were submitted to total RNA extraction and high throughput sequencing. Identity of 99% with Amaranthus leaf mottle virus (AmLMV, *Potyvirus*) was observed. This is the first report of AmLMV in both *Impatiens walleriana* and the Americas.

Keywords Ornamental species · Balsaminaceae · *Potyvirus* · Next-generation sequencing

The native range of *Impatiens walleriana* (Balsaminaceae), popularly known as busy lizzie or impatiens, extends from southeastern Kenya to southern tropical Africa. *I. walleriana* has been widely cultivated mainly for its variety of flower shapes and colours, early flowering, flower petal textural patterns, flower size, resistance to diseases and pests, and mainly, resistance to extreme sunlight (Ghanbari et al. 2019). It is a subshrub with high reproductive capacity that grows primarily in the Wet Tropics, and is well adapted to the environmental conditions in Brazil, where it became naturalised (i.e. spontaneous) in the Atlantic Forest (Souza and Lorenzi 2012; Ghanbari et al. 2019; POWO 2023).

Although impatiens plants are hosts of several viruses (Cho et al. 2017; Diningsih et al. 2020), in Brazil only cucumber mosaic virus (CMV) and an unidentified potyvirus have been described in *I. walleriana* in Brasilia (Federal District) and Paraná state. Tomato mosaic virus (ToMV) infection has also been reported in *I. hawkeri* (Kitajima 2020).

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This study aimed to identify the virus associated with mosaic, blistering and leaf deformation, and flower colour break symptoms in *I. walleriana* (Imp-87 isolate) from Itu (GPS coordinates: 23°15×51″ S 47°17×57″ W), São Paulo state, Brazil (Fig. 1). Samples were analysed by transmission electron microscopy (morphology of viral particles and presence of cytoplasmic inclusions), biological (mechanical transmission) and serological tests (ELISA), and whole-genome sequencing. Leaf extracts from symptomatic plants were negatively stained with 1% uranyl acetate. For ultrastructural observations, small leaf fragments were fixed and processed by transmission electron microscopy (Kitajima and Nome 1999). Examinations were conducted using a JEOL JEM 1011 TEM (JEOL, Akishima, Japan) transmission electron microscope, and images were recorded digitally. Ultrathin sections cut from symptomatic impatiens leaf tissues revealed type I in Edwardson's classification (Edwardson and Christie 1978), in an overview of a cytoplasmic area taken by a large number of scroll-like cylindrical inclusions (CI), in longitudinal and cross sections (Fig. 2).

A symptomatic impatiens leaf sample was evaluated by DAS-ELISA (in triplicate wells) using a potyvirus group antiserum (Agdia, Elkhart, USA), according to the manufacturer's instructions, and PTA-ELISA for CMV with polyclonal antiserum. Symptomatic *I. walleriana* leaf samples were positive for potyvirus, and negative for CMV.

Total RNA from *I. walleriana* leaves was extracted with TRIzol (Life Technologies, Carisbad, CA, USA), according to the manufacturer's instructions. RT-PCR was performed using a pair of primers that amplify part

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Fig. 1 *Impatiens walleriana* Hook.f. plant with mosaic, blistering and leaf deformation and flower colour break from a public garden in Itu, Brazil

of the potyvirus polyprotein (Gibbs and Mackenzie 1997). Amplicons of around 1,500 bp (GenBank accession number OQ919270) were directly sequenced in both forward and reverse directions using the PCR primers in an ABI PRISM377 sequencer (Applied Biosystems). Blast X analysis indicated the best matches with sequences corresponding to the partial NIb regions and full-length protein coat of potyviruses. The highest identity values (> 91%) were obtained with sequences of Amaranthus leaf mottle virus (AmLMV) isolated from *Amaranthus* sp. from

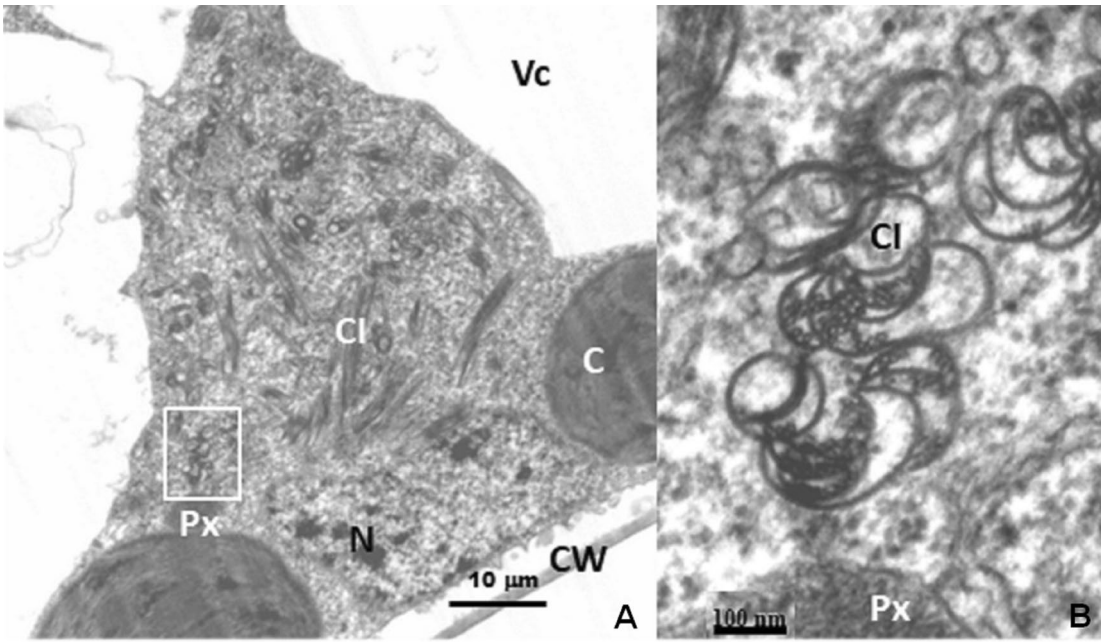

Fig. 2 Transmission electron micrograph of ultrathin sections of symptomatic Impatiens 87 leaf tissue. **A**. An overview of a cytoplasmic area taken by a large number of (CI), type I in Edwardson's classification, in longitudinal and cross sections. **B**. Detail of the marked area in A, showing cross sectioned CI. C- chloroplast; CW- cell wall; N- nucleus; Px- peroxisome; Vc- vacuole

France and Italy (GenBank accession numbers MN709786 and OL584351).

An extract from fresh symptomatic leaves of *I. walleriana* was obtained by grinding in a mortar with 0.05 M phosphate-buffered saline (PBS), pH 7.4, containing 0.1% mercaptoethanol. Five plants of the species *Chenopodium giganteum* (sin. *C. amaranticolor*), *C. murale*, *C. quinoa*, *Gomphrena globosa* (Amaranthaceae), *Datura stramonium*, *Nicotiana glutinosa*, and *N. tabacum* Sansum, (Solanaceae) were mechanically inoculated. Imp 87 failed to produce any local or systemic symptoms in test plants, except *C. quinoa*, which responded with chlorotic lesions on the inoculated leaves (local symptom) of all 5 plants, unlike what was reported for the AmLMV isolate from *Amaranthus viridis* from Spain (AL isolate), which also exhibited systemic symptoms (Segundo et al. 2007). The AL isolate also induced local symptoms in *C. amaranticolor*.

To confirm the identity of the virus, high-throughput sequencing (HTS) using HiSeq 2500 technology (2×150 nt paired-end reads) (Illumina, San Diego, CA, USA) was also performed. Reads were assembled with SPAdes (Bankevich et al. 2012), and contigs comprising viral sequences were identified using BLASTx, BLASTn and custom plant viral genome databases retrieved from NCBI virus (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/). After contig analysis, a sequence of 9,228 bp, corresponding to P1, HC-Pro, P3, 6K1, CI, 6K2, NIa-Vpg, NIa-Pro, NIb, CP and PIPO proteins, was obtained and made available on GenBank under accession number OR284325. The nucleotide and amino acid sequences corresponding to the polyprotein of the Imp 87 isolate showed 99% identity with AmLMV isolates.

AmLMV was described as a potyvirus isolated from *Amaranthus deflexus* in Italy (Lovisolo and Lisa 1976) and serologically related to bean yellow mosaic virus and plum pox virus (Rajeshwari et al. 1983). The virus has only been isolated from *Amaranthus* species in Italy, Spain, Morocco and France (Lovisolo and Lisa 1976, 1979; Casetta et al. 1986; Segundo et al. 2007; Moury and Desbiez 2020). Although there is no genome sequence of AmLMV isolated from host plants other than those of the genus *Amaranthus* available on GenBank, its occurrence in *Cirsium arvense* has been previously described (Casetta et al. 1986). As such, this study is the second report of AmLMV in a species other than *Amaranthus*, the first report of AmLMV in the Americas, and the first AmLMV polyprotein complete sequence of an isolate collected outside Europe. To date, the occurrence of AmLMV has been described in the genera *Amaranthus* (Amaranthaceae) and *Cirsium* (Asteraceae), and now in *Impatiens* (Balsaminaceae). These families belong to a monophyletic group within the core eudicots (APG 2016). However, speciation of the studied plant species started well before the initial potyvirus radiation about 7,000 years ago, meaning that these changes correspond to host jumps rather than host–virus co-divergences (Gibbs and Ohshima 2010; Moury and Desbiez 2020). Although there is no report of AmLMV seed transmission, the virus may have entered Brazil in seeds imported from infected *A. deflexus*, since *C. arvense* is considered a quarantine pest absent in Brazil. Aphids may have transmitted the virus to *I. walleriana* plants, initiating a compatible interaction. Given that impatiens is cultivated as an ornamental, in addition to invading crops and being common in forest borders of urban Atlantic Forest areas, AmLMV may damage economically important crops such as spinach, beets and New Guinea impatiens, as well as native Amaranthaceae species.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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